AN EXPERIMENTAL ANALYSIS OF THE MICRO-NEUROANATOMY OF THE CENTRAL NERVOUS SYSTEM

Biophysical Research Laboratory
University of Illinois
Urbana, Illinois

Grant No. NsG 195-62

National Aeronautics and Space Administration

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Period covered:

30 September 1964 through 1 April 1965

N65-85962
(ACCESSION NUMBER)
6
(PAGES)
OR 63614
(NASA CR OR TMX OR AD NUMBER)
FACILITY FORM 602
(THRU)
(CODE)
(CATEGORY)

1 INTRODUCTION

1.1 Statement of problem. Application of the quantitative anatomical methods developed under the present contract grant for the determination of neural organization of structures of the central nervous system has been expanded and the emphasis has been placed on studies of the vestibular nuclei in the brain stem of the squirrel monkey. The primary objectives of the research, in terms of developing reliable methods for obtaining reproducible data clearly demonstrating quantitative structural organization in brain systems and the ability to derive the "complete" neuron circuitry by such methods, have been satisfactorily demonstrated in the limbic system of the cat.

The deeper insight into the anatomical relations which the present research has provided indicates definitely that it is desirable to pursue investigations in the time relations obtaining between the complex configurations of the dynamic events characteristic of brain operation as an adjunct to the quantitative elucidation of function. The interruption of information along neural pathways by means of focused ultrasound in combination with stimulation means would be a desirable research direction.

1.2 Summary of present activities. Preliminary work on the vestibular system in the squirrel monkey is in progress. Ventriculography procedures have been adapted to this animal and the results have indicated that a satisfactory localization of anatomically significant structures will be possible. The ultrasonic irradiation of vestibular nuclei in the squirrel monkey for the purpose of establishing dosage and placement accuracy has been started. Behavior changes and functional deficits are noted as means of evaluating lesion placement prior to histological studies. Unmodified squirrel monkey brains have been prepared for histological and cytological examination. These brains will provide data on neuron populations in "normal" squirrel monkey brains.

Instrumentation for possible direct stimulation of small localized regions anywhere within the brain (insertion of electrodes into the brain mass is unnecessary with this equipment) has been completed recently.

1.3 Communications. A paper which covers a great deal of the methodology involved in the data acquisition processes and also contains some data on neuron counts and neuron distributions was published in the Journal of the Acoustical Society of America (36, 1795-1835 (1964)).

A series of papers have been presented disclosing the quantitative anatomical method and results:

a. "Mammillary Complex of Cat Brain--Quantitative Organization" at American Association of Anatomists 78th Annual Meeting, Miami Beach, Florida, April, 1965.

b. "Quantitative Neuroanatomic Relations in the Mammillary Complex of Cat Brain" at American Academy of Neurology, 17th Annual Meeting, Cleveland, Ohio, April, 1965.

c. "Ultrasound in Biomedical Engineering" at Annual Spring Seminar Series, University of Texas, Austin, Texas, April, 1965.

d. "Brain Circuitry" at Biomedical Engineering Seminar, Rice University, Houston, Texas, April, 1965.

e. "Ultrasound as a Research Tool in Biomedical Studies" at Medical Seminar at Bowman-Gray School of Medicine, Winston-Salem, N.C., May, 1965.

f. "Methods for Completely Deciphering the Neuronal Circuitry of Brain Structures". To be published in the Proceedings of the 6th International Conference on Medical Electronics and Biological Engineering. Tokyo, Japan.

g. A very extensive paper entitled "Mamillary Nuclei and Associated Complex of Cat - Quantitative Analysis" submitted to the Journal of Anatomy of London. This paper has been returned for some revisions.

2. WORK IN PROGRESS.

2.1 Instrumentation and technique (anatomy). As outlined in the proposal, the squirrel monkey as an experimental animal provides a number of unique advantages to this study. A colony of healthy young animals has been established. Problems of diet, medications and special care have been resolved. Postoperative animals have required intensive care supervision. Comprehensive sterile technique is used for the surgery required on these animals. Highest standards of animal care and humane treatment are employed.

Ventriculography procedures have been performed on a number of squirrel monkeys in order to standardize the procedure for fourth ventricle visualization which is valuable in localization involving the vestibular nuclei. This ventriculography procedure utilizes a radio-opaque dye, Conray, produced by the Mallinckrodt Chemical Company, St. Louis, Mo. This is the same material which we have used on hundreds of cats. The animals have recovered from the

ventricular procedure extremely well and have shown no deficits in behavior or function.

Since the ultrasound method of lesion production will be used in studies involving degeneration of neural tissue, a number of monkeys have been prepared surgically for this procedure. This involves a bone flap preparation which is readily removable for the ultrasonic irradiation procedures. The squirrel monkey tolerates this procedure satisfactorily.

Unilateral ultrasonic irradiation of the lateral and medial vestibular nuclei of a squirrel monkey has been accomplished. Various neurological deficits were noted which suggest that the lesion placement was accurate. Marked nystagmus and ataxia were observed immediately following recovery from anesthesia. The symptoms of nystagmus persisted for about two weeks. A postural deficit in the form of a marked rotation of the sagittal plane of the head toward the right persists in excess of two weeks.

The relation between nucleolus diameter and axon length has been suggested. This concept has proved to be useful in establishing subpopulations within the mammillary nuclei of the cat. The correlation between subpopulations determined by this method with retrograde and transneuronal depopulation studies has been demonstrated. The nucleoli diameters in the mammillary complex of the cat range in size from 2μ to 3μ ; consequently, the measurement of such dimensions requires very accurate methods.

In order to obtain dimensional stability of sufficient accuracy on the nucleoli diameters of the neuron populations of interest it was found necessary to control the temperature of the glass slide with the mounted and stained brain section and the microscope temperature to within limits not readily obtainable in the normal open room. A temperature range of $\pm$ 0.3°C was found to give the necessary repeatibility for such work. Two temperature-and humidity-controlled housings have been constructed, tested, and used with the microscope for nucleolus diameter measurements. These housings contain the microscope used for the measurements and they are provided with double wall flexible ports by means of which the microscope stage and focusing adjustments can be made without air exchange and unnecessarily large heat exchange to the outside. These housings have been very satisfactory for the nucleolus diameter measurements -- measurements reproducible to $\pm$ 10 micro-μ have been made on test slides.

A decitrack encoder has been attached to each of the microscopes in the temperature controlled housings. These encoders, through appropriate interface equipment, are used to automatically record the angular positions of the micrometer eyepiece apparatus, thereby eliminating the necessity of manual readout and recording of the nucleolus diameter. The resulting saving in time from the use of these items of data processing is considerable (a gain in time of 3 to 1 has been realized), but in order to further speed the total data processing, a system is being initiated whereby data taken from the encoder on the microscope will be recorded on tape suitable for feeding directly into one of the digital computers available at the University of Illinois. This method will eliminate many of the human errors which occur in desk calculator manipulations and transferral of numbers.

Additional personnel is being trained to make nucleolus diameter measurements; this training involves considerable effort and time since each new trainee must demonstrate ability to maintain the desired degree of accuracy and repeatibility.

With the microscope measurement and readout systems now available, one set of sections for the lateral mammillary nucleus of Cat 993C has been measured in terms of the nucleolar diameters. Before any elaborate data processing in terms of generating distribution functions for subpopulations in this nucleus is attempted there will be one or two more sets of measurements. With this amount of data available for a given nucleus it would appear that the critical analysis of subpopulations would achieve a high degree of accuracy.

In order to facilitate the measurements on the nucleolar diameters a thionine staining technique has been evolved which enhances the nucleolus boundary. The evolved technique produces stained sections which are very stable despite prolonged exposure to light. It is extremely important that the stained nucleoli remain constant over the time periods needed to accumulate the dimensional data. The use of phase optics has further improved the reliability of nucleoli measurements.

2.2 Instrumentation and technique (functional study). The high power driver for providing more than 100 kw of electrical power in the frequency range of 1 to 10 Mc has been completed and checked to 70 kw output. This driver provides a part of the instrumentation necessary to obtain acoustic power levels of sufficient magnitude to study effects such as the possibility of direct brain stimulation without the introduction of probe electrodes. Some preliminary work

on cat and monkey brains has been done with the combination of rf conduction current and ultrasound of the same frequency. There has as yet been no serious attempt to provide direct brain stimulation since technique and instrumentation problems remain to be resolved.

A large transducer employing a 5" diameter X-cut quartz plate has been fitted with a lens for focusing the sound beam. Field plots have been made at 1 Mc and 3 Mc. The 3 Mc plots indicate that the sound field at the focus is appropriate for irradiation or stimulation experiments at this this frequency. The 1 Mc plots indicate some anomalies which will require investigation.